REMARKS

Reconsideration and allowance are respectfully requested.

Claims 1-25 and 40-42 are pending. Non-elected claims 26-39 were withdrawn from consideration by the Examiner. Applicants cancel the non-elected claims without prejudice to future prosecution of that subject matter.

Specification

The Examiner required Applicants to amend the disclosure to include "essential material" incorporated by reference but she did not specify what was allegedly being improperly incorporated by reference. No particular portion of Applicants' specification was cited in the requirement.

Clarification is requested because it is not clear to Applicants what the Examiner considers "essential material" or an improper incorporation by reference.

35 U.S.C. 112 – Written Description

The specification must convey with reasonable clarity to persons skilled in the art that applicant was in possession of the claimed invention as of the filing date sought. See *Vas-Cath v. Mahurkar*, 19 USPQ2d 1111, 1117 (Fed. Cir. 1991). But the Patent Office has the initial burden of presenting evidence or a reason why persons of ordinary skill in the art would not have recognized such a description of the claimed invention in the original disclosure. See *In re Gosteli*, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989).

Claims 1-25 were rejected under Section 112, first paragraph, because it was alleged that they contain "subject matter which was not described in the specification in such a way as to reasonably convey to any person skilled in the art to which it pertains, or with which it is most nearly connected, at the time the application was filed, that the inventor, at the time the application was filed, had possession of the claimed invention." Applicants traverse.

The main objection appears to be that Applicants allegedly teach only one means for altering melusin expression. Although their work has concentrated on homologous recombination to inactivate melusin expression, Applicants teach other embodiments of

the invention (i.e., "species") supporting the generic claims. In particular, a "transgenic animal" within the scope of the claims is defined at page 11, line 34, to page 12, line 5, of Applicants' specification. The invention is not limited to inactivating the melusin gene by homologous recombination. Two additional non-limiting examples are taught at page 12, lines 6-17, of the specification: antisense inhibition and RNA interference. They represent a constructive reduction to practice of two other embodiments within the scope of the invention. It is well known in the art that many non-human animal species other than mouse are amenable to such genetic technology. A specification need not teach, and preferably omits, what is well known in the art. See *Hybritech v. Monoclonal Antibodies*, 231 USPQ 81, 94 (Fed. Cir. 1986). Therefore, the description of a "transgenic animal" in Applicants' specification satisfies the Section 112 requirement.

The "core structure and function" necessary to make and use the invention is the melusin gene. Melusin was well-characterized by Brancaccio et al. as demonstrated by their published peer-reviewed article (J. Biol. Chem. 274:29282-29288, 1999; listed on PTO-1449) and the specification need not teach what was disclosured there. See *Hybritech* at 94. cDNA and genomic DNA fragments of the mouse melusin gene are taught at page 12, lines 33-35, of the specification. Actual reduction to practice of melusin-null mice is taught in Example 1 of the specification. Clearly, the possession of the genomic fragments of melusin and the ability to achieve homologous recombination teach the skilled artisan how to insert mutations in any part of the melusin gene. For example, a truncated melusin protein with a deletion of the beta1 integrin binding domain could be used. A representative number of species (i.e., DNA constructs) to support their generic recitation in Applicants' claims is therefore available from the sequences taught. And as noted above, the teaching of alternative technologies such as antisense inhibittion and RNA interference to make transgenic animals which have altered melusin expression are representative of the broad genus claimed by Applicants. Melusin's central function in controlling the hypertrophic response of the heart is taught in Fig. 10 and at page 10, line 30, to page 11, line 2, of the specification. Therefore, the description of melusin's "core structure and function" in Applicants' specification satisfies the Section 112 requirement.

It was objected in the Action at page 4 that a "description of all cells that naturally do not have melusin expression is lacking." But Applicants' specification states at page 13, lines 26-30, and in Fig. 4 that melusin is normally expressed in heart and skeletal muscle cells. Cells that do not "naturally" express melusin would not be analyzed or otherwise discussed because such cell types do not show altered expression when they do not normally express melusin. Such tissue-specific expression is an advantage for a drug target as taught at page 10, lines 25-30, of the specification. But it is noted that muscle cells which have an altered melusin expression are not the only cells that have utility in the invention. For example, embryonic stem cells (as well as pluripotent and multipotent cells), sperm, oocytes, and fertilized oocyes which might not transcribe and translate melusin are useful for preparing non-human transgenic animals. Therefore, the description of "cells" in Applicants' specification satisfies the Section 112 requirement.

Finally, Brancaccio et al. (J. Biol. Chem. 274:29282-29288, 1999) discloses what was well known in the art. Rat, mouse and human sequences were isolated. Deduced amino acid sequences are compared between human and mouse melusin in Fig. 1. Cells that express or do not express melusin are shown in Fig. 2. Melusin's function of binding beta1 integrin was assigned to a particular protein domain in Tables I-II and the Ca^{2+} dependence of binding was shown in Fig. 5. As cited above, a specification need not teach, and preferably omits, what is well known in the art as demonstrated by Brancaccio et al. See *Hybritech* at 94.

Withdrawal of the written description rejection made under Section 112, first paragraph, is requested because the specification conveys to a person skilled in the art that Applicants were in possession of the claimed invention as of the filing date.

35 U.S.C. 112 – Enablement

The Patent Office has the initial burden to question the enablement provided for the claimed invention. M.P.E.P. § 2164.04, and the cases cited therein. It is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain <u>why</u> it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with

the contested statement. *In re Marzocchi*, 169 USPQ 367, 370 (C.C.P.A. 1971). Specific technical reasons are always required. See M.P.E.P. § 2164.04.

Claims 1-25 were rejected under Section 112, first paragraph, because it was alleged that they contain "subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention." It was also alleged that "[t]he specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims." Applicants traverse for the following reasons citing the Examiner's points by references to page number of the Office Action where they are found.

- **Page 6** - *Generation and study of heart hypertrophy, dilation and failure in transgenic animals* are well known to the skilled artisan. They have been the object of several textbooks, that are universally considered state-of-the-art by cardiologists:
  - BRAUNWALD'S HEART DISEASE: A TEXTBOOK OF CARDIOVASCULAR MEDICINE
    Edited by Braunwald E, WB Saunders, Philadelphia, PA
    First edition, 1980 – Fourth edition, 1999 – Seventh edition, 2004
  - MOLECULAR BASIS OF CARDIOVASCULAR DISEASE
    Edited by Chien KR, WB Saunders, Philadelphia, PA
    First edition, 1999 – Second edition, 2004

- **Page 6** - *The techniques to generate hypertensive conditions in transgenic animals such that they develop heart hypertrophy, dilation and failure* have been extensively described since early 1990 both in laboratory handbooks and publications in top quality scientific journals:
  - Strategies for studying cardiovascular disease in transgenic and gene-targeted mice. P Doevendans, JJ Hunter, G Lembo, K Wollert, KR Chien

In: STRATEGIES IN TRANSGENIC ANIMAL SCIENCE
Edited by Monastersky GM and Robl JM, Washington, DC, 1995

- The Autonomic Nervous System and Catecholamines in Normal Blood Pressure Control and in Hypertension. Goldstein DS, Kopin IJ
  In: HYPERTENSION: PATHOPHYSIOLOGY, DIAGNOSIS AND MANAGEMENT
  Edited by Laragh JH, Brenner BM, Raven Press, New York, NY, 1990
- Segregation of Atrial-Specific and Inducible Expression of an Atrial Natriuretic Factor Transgene in an in vivo Murine Model of Cardiac Hypertrophy. HA Rockman, RS Ross, AN Harris, KU Knowlton, ME Steinhelper, LJ Field, J Ross Jr, KR Chien
  Proc Natl Acad Sci USA 88:8277-8281, 1991
- Transition from Compensatory Hypertrophy to Dilated, Failing Left Ventricles in Dahl Salt-Sensitive Rats. Inoko M, Kihara Y, Morii I, Fujiwara H, Sasayama S
  Am J Physiol 267:H2471-2482, 1994
- Angiotensin II Stimulation of Left Ventricular Hypertrophy in Adult Rat Heart: Mediation by the AT1 Receptor. Dostal DE, Baker KM
  Am J Hypertens 5:276-280, 2001
- Molecular and Cellular Mechanisms of Angiotensin II-Mediated Cardiovascular and Renal Diseases. Kim S, Iwao K
  Pharmacol Rev 52:11-34, 2000

- **Page 6** - *The techniques to study heart hypertrophy, dilation and failure in transgenic mice* are well known to the skilled artisan since they have been extensively described, both in the general textbooks mentioned above and in publications in top quality scientific journals:
  - Physiological Assessment of Complex Cardiac Phenotypes in Genetically Engineered Mice. Christensen G, Wang Y, Chien KR
    Am J Physiol 272:H2513-2524, 1997

- Exploring the Role of the Beta-Adrenergic Receptor Kinase in Cardiac Disease using Gene-Targeted Mice. Koch WJ, Rockman HA
  Trends Cardiovasc Med 9:77-81, 1999
- Genetically Engineered Mice: Model Systems for Left Ventricular Failure. Kadambi VJ, Kranias EG
  J Card Failure 4:349-61, 1998
- Cardiovascular Phenotyping in Mice. Doevendans PA, Daemen MJ, de Muinck ED, Smits JF
  Cardiovasc Res 39:34-49, 1998

- **Page 6** - *The protocols for screening compounds pharmacologically active in prevention and or treatment of heart dilation and failure* are highly variable depending on the nature of the compound to be tested. Melusin null mice, in fact, can be useful to test and validate both novel compounds and drugs with known activity. The routes of drug administration can be intraperitoneal, intradermal, intramuscular, intravein, intracardiac injection, controlled continuous release by osmotic pumps, and oral or rectal administration. The treatment can be acute or, more likely, chronic. The efficacy of the compound can be evaluated by conventional techniques used to measure heart function and remodeling such as those described in Examples 2 and 3 of the specification. All the techniques mentioned above for testing pharmacological activity and toxicity of drug compound are well known to the skilled artisan and are described in detail in laboratory handbooks such as:
  - DRUG DISCOVERY AND EVALUATION: PHARMACOLOGICAL ASSAYS, SECOND EDITION
    Edited by Vogel HG, Springer Verlag, New York, NY, 2002

- **Page 6** - *The techniques to generate transgenic animals* have been extensively described since early 1990 and are well known to the skilled

artisan and fully described both in laboratory handbooks and publications in top quality scientific journals.

Different transgenic animal species have been obtained including: mouse, rat, pig, caw, goat, sheep, rabbit, chicken, frogs, salmon, zebrafish, drosophila and Caenorhabditis elegans.

- TRANSGENIC ANIMALS GENERATION AND USE
  Houdebine LM, Harwood Academic Publisher, 1997
- Transgenic Production of a Variant of Human Tissue-Type Plasminogen Activator in Goat Milk: Generation of Transgenic Goats and Analysis of Expression. Ebert KM, Selgrath JP, DiTullio P, Denman J, Smith TE, Memon MA, Schindler JE, Monastersky GM, Vitale JA, Gordon C
  Bio/Technology 9:835-838, 1991
- Transgenic Pigs Produce Functional Human Factor VIII in Milk. Paleyanda RK, Velander WH, Lee TK, Scandella DH, Gwazdauskas FC, Knight JW, Hoyer LW, Drohan WN, Lubon H
  Nat Biotechnol 15:971-975, 1997
- Niemann H, Halter R, Carnwath JW, Herrmann D, Lemme E, Paul D. Expression of Human Blood Clotting Factor VIII in the Mammary Gland of Transgenic Sheep. Transgenic Res 8:237-47, 1999
- van Berkel PH, Welling MM, Geerts M, van Veen HA, Ravensbergen B, Salaheddine M, Pauwels EK, Pieper F, Nuijens JH, Nibbering PH. Large Scale Production of Recombinant Human Lactoferrin in the Milk of Transgenic Cows. Nat Biotechnol 20:484-487, 2002
- Krimpenfort, P. *et al.* Generation of Transgenic Dairy Cattle Using '*In Vitro*' Embryo Production. Bio/Technology 9:844-847, 1991
- Amaya, E. and Kroll, K.L. A Method for Generating Transgenic Frog Embryos. In: METHODS IN MOLECULAR BIOLOGY, Edited by Sharpe P, Mason I. Humana Press, Totowa, NJ, 97:393-414, 1999

- **Pages 8 and 9** - *The two papers cited describe the consequences of beta1 integrin mutations in cardiac structure and function.*
  - ➢ Fassler R, Rohwedel J, Maltsev V, Bloch W, Lentini S, Guan K, Gullberg D, Hescheler J, Addicks K, Wobus AM. Differentiation and integrity of cardiac muscle cells are impaired in the absence of beta 1 integrin. J Cell Sci 109:2989-2999, 1996

The authors analyzed cardiac differentiation in beta1 integrin deficient embryonic stem cells concluding that beta 1 integrin is important for normal cardiogenesis both during embryonic stem cell differen-tiation in vitro and in vivo. Moreover, the sarcomeric architecture is incomplete and disarranged in the absence of beta 1 integrin. The functional consequences of beta1 integrin knockout are, thus, very different from those observed in melusin null mice where the absence of melusin does not affect heart function in basal conditions (see Fig. 5 of Applicants' specification) implying no defect in cardiomyocyte differentiation nor in sarcomeric architecture. See Brancaccio et al. (Nat. Med. 9:68-75, 2003).

- ➢ Shai SY, Harpf AE, Babbitt CJ, Jordan MC, Fishbein MC, Chen J, Omura M, Leil TA, Becker KD, Jiang M, Smith DJ, Cherry SR, Loftus JC, Ross RS. Cardiac myocyte-specific excision of the beta1 integrin gene results in myocardial fibrosis and cardiac failure. Circ Res 90:458-464, 2002

The authors generated a mouse that lacks beta1 integrin selectively in the heart ventricles. This genetic mutation cause extensive accumulation of fibrosis in ventricles and dramatic functional defects that lead to spontaneous failure at six months of age. Here, the phenotype resulting from beta1 integrin inactivation is not comparable to that observed in melusin null mice which show no functional heart defects in basal condition and do not develop heart failure spontaneously (see Fig. 5 of Applicants' specification). See Brancaccio et al. (Nat. Med. 9:68-75, 2003).

In conclusion melusin, in binding to integrin beta1 in cardiomyocytes, mediates a novel and specific function downstream of integrins. More specifically it activates the hypertrophy signaling pathways in response to mechanical overload without affecting the basal function of these receptors as indicated by the results reported in Applicants' specification and published in Brancaccio et al. (Nat. Med. 9:68-75, 2003).

Also at page 9 of the Office Action: "the claimed invention . . . is considered unpredictable" This allegation is incorrect because altered melusin expression and its physiological function (e.g., development of pathology after exposure to hypertensive conditions) comes from the results disclosed in Applicants' specification. Based on our results showing that melusin ablation causes heart dilation and failure upon pressure overload, we teach that melusin overexpression can protect heart from pressure overload. This is confirmed by the result reported in the publication of DeAcetis et al. (Circ Res 96:1087-1094, 2005). Their results thus do not contradict Applicants' claims, but rather confirm and extend it.

- **Page 10** - *Genetic approaches to generate transgenic animals with altered melusin expression RNA interference or antisense.*

The concerns raised in the article by Houdebine LM (J Biotechnol 98:145-160, 2002) are simply understood as considerations of caution that should be taken into account in any experimental system where exact conditions need to be defined and selected for their efficacy. The document cited is a review of the most commonly available technologies to generate transgenic animals. It is not a specific criticism of antisense technology.

The argument that antisense oligonucleotides are sequestered in endosomes (Hughes MD, Hussain M, Nawaz Q, Sayyed P, Akhtar S. Drug Dis Today 6:303-315, 2001) does not apply to the generation of transgenic animals in which the antisense sequence is synthesized within the cell by a

transgene integrated into the genome as taught in Applicants' specification. In fact, the oligonucleotide does not have to enter the cell trough the plasma membrane via the endocytic pathway as was required when antisense oligonucleotides were used as drugs. The document cited is a review of drug delivery systems instead of the transgenic approach.

- **Page 12** - *The techniques to transiently modify the gene expression in transgenic mice* such as the tetracycline system are well known to the skilled artisan and are widely described in the literature (reviewed by Houdebine LM. J Biotechnol 98:145-160, 2002). Also, neurodegenerative disease is not discussed in Applicants' specification.

- **Page 13** - "However the applicant submit that these phenotypes are not exhibited when using hypertensive drugs.
  In Example 3, Applicants teach that "the inventors also tested the cardiac response in melusin null mice after chronic administration of phenylephrine or angiotensin II at sub-pressor doses which do not increase blood pressure." Indeed, phenylephrine or angiotensin II are hypertensive drugs but in these specific experimental conditions, the doses used do not cause increased blood pressure. Under these conditions, the melusin null heart undergoes a normal hypertrophy response. This does not contradict the fact shown in Applicants' specification that melusin null mice have a defective response when exposed to conditions that induce hypertension.

- **Page 13 bottom** - The definition of heart pathologies is totally superfluous as it is common knowledge to any cardiologist (i.e., a skilled artisan).

Withdrawal of the enablement rejection made under Section 112, first paragraph, is requested because it would not require undue experimentation for a person of skill in the art to make and use the claimed invention.

35 U.S.C. 112 – Definiteness

Claims 18-19 and 23 were rejected under Section 112, second paragraph, as being allegedly indefinite because "since the claim does not set forth any steps involved in the method/process, it is unclear what method/process applicant is intending to encompass." Applicants traverse because the claims recite active, positive steps involved in the methods.

Applicants request withdrawal of the Section 112, second paragraph, rejection because the pending claims are clear and definite.

35 U.S.C. 101 –Utility

Claims 18-19 and 23 were rejected under Section 101 because "the claimed recitation of a use, without setting forth any steps involved in the process" allegedly results in an improper definition of a process. Applicants traverse because the claims set forth active, positive steps involved in the methods.

Withdrawal of the Section 101 rejection is requested.

Conclusion

Having fully responded to all of the pending objections and rejections contained in this Office Action, Applicants submit that the claims are in condition for allowance and earnestly solicit an early Notice to that effect. The Examiner is invited to contact the undersigned if any further information is required.

Respectfully submitted,

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